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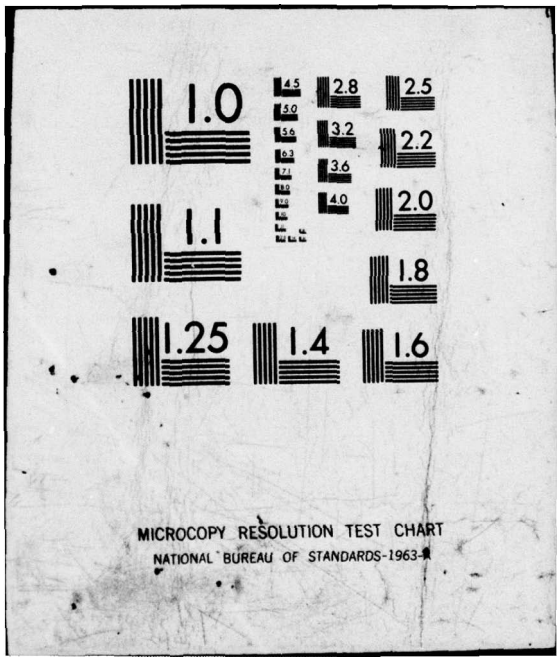
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DEVELOPMENT AND CHARACTERIZATION OF ATTENUATED LIVE INFLUENZA VIRUS VACCINES FOR USE IN MAN

FINAL REPORT

by

H. F. Maassab, Ph.D.

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Derivation and characterization of a new cold recombinant, AA-CR19, was accomplished. The line was derived by recombination at 25°C between the attenuated and temperature sensitive cold variant A/AA/6/60-H3N2. Twenty clones were isolated and analyzed biologically and immunologically. One clone with the desired properties was used to produce an experiemental vaccine lot for use in man.

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The cut-off temperature of different cold mutant lines in primary chick kidney followed a preditable pattern with some viruses having 37°C and some 38°C cut-off temperature, however, one recombinant AA-CR13 appears to grow equally well at 25°C and 39°C. The dissociation between temperature sensitivity and cold adaptation was observed with the cold recombinant AA-CR13. The line appears to grow at 39°C but it did retain its growth at 25°C and its loss of virulence when administered to ferrets and mice.

Transfer of cold genes was accomplished by recombination at 33°C instead of 25°C. The data obtained clearly establish that cold genes are acquired by recombination at 33°C, and thus spontaneous mutation can be ruled out as the mechanism of emergence of cold recombinants.

Collaborative studies with Drs. Spring, Murphy and Chanock at NIAID and Dr. A.P. Kendal at CDC in Atlanta, in comparing the cold variants and their recombinants with the ts mutants have provided the following: 1) Some of the cold variants and recombinants share a lesion with complementation group I of the 5 FU ts mutants and three, had additional ts lesions, 2) All viruses evaluated for replication in the lungs and nasal turbinates, appear to be attenuated, 3) Some of the cold mutants did not revert and were genetically stable, 4) Cold adaptation was transferred and can be dissociated from ts property by recombination at 33°C in primary rhesus monkey kidney cells, and 5) The RNA pattern of one cold recombinant and its parents have furnished preliminary results which can correlate the specific area of the genome of virus to the cold adaptation process.

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SUMMARY

Development and characterization of a cold recombinant AA-CR19 was accomplished. The line was derived by recombination at 25°C between the attenuated and temperature sensitive cold variant A/AA/6/60-H2N2 and the new epidemic strain of type A influenza virus A/Victoria/3/75-H3N2. Twenty clones were isolated and analyzed biologically and immunologically. One clone with the desired properties was used to produce an experimental vaccine lot for use in man.

The cut-off temperature of different cold mutant lines in primary chick kidney followed a preditable pattern with some viruses having 37°C and some 38°C cut-off temperature, however, one recombinant AA-CR13 appears to grow equally well at 25°C and 39°C. The dissociation between temperature sensitivity and cold adaptation was observed with the cold recombinant AA-CR13. The line appears to grow at 39°C but it did retain its growth at 25°C and its loss of virulence when administered to ferrets and mice.

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I. Derivation of a cold recombinant (AA-CR19) to the new strain A/Victoria/3/75-H3N2.

A new type A of influenza virus (A/Vict/3/75-H3N2) emerged in early 1976 in the U.S. The strain which showed minor changes from the current prototype strain (A/Port Chalmers/1/75-H3N2) was received from Dr. Dowdle of CDC in Atlanta. Recombination at 25°C in primary chick kidney cells (PCKC) as illustrated in the flow diagram enclosed was used to derive appropriate attenuated clones for analysis as outlined in the new contract proposal submitted September 1976 for consideration. The attenuated cold mutant (A/AA/6/60-H2N2) served as the donor strain since it has been completely characterized in terms of genetic stability (1,2,3) and acceptability as a live avirulent and immunogenic strain in man. Mixed infections of PCKC was initiated at 25°C using 5 PFU/cell with the cold variant A/AA/6/60-H2N2 and the wild strain A/Victoria/3/75-H3N2. After 72 hrs, second and third passages were made at five day intervals with antisera oriented against the cold variant. A fourth passage without antiserum was carried out in embryonated eggs held at 25°C. Parallel passage of wild and cold parents failed to yield virus, demonstrating that the antiserum employed had been effective in suppressing growth of the cold parent and that final yield of the mixed infection series was the product of genetic recombination rather than the result of spontaneous emergence of a cold mutant. The fourth passage of the mixed infection was plaque titrated in PCKC at 25°C and 20 clones isolated for biologic and immunologic characterization. One clone with the appropriate markers was sent to Michigan Department of Health for production of an experimental vaccine lot to be used in man.

II. Determination of the cut-off temperatures of cold mutants derived by 3 different methods.

The determination of cut-off temperature in replicate cultures of primary chick kidney cells (PCKC) of the different lines of cold mutants is illustrated in Table 1. The plating efficiency exhibited a predictable pattern. It is apparent that the three methods used for selecting of cold mutants furnished similar pattern of growth at the different temperatures of 37°, 38° and 39°C. The one exception is the cold recombinant AA-CR13 which appears to grow at 39°C. The titer of each of the parental wild parents was equal at all 4 temperatures. Similar results were obtained at NIH in primary rhesus monkey kidney cells (4) using the same strain of influenza virus shown in Table 1. Thus, it is safe to assume that the growth characteristic of these cold mutants appears to be stable and is not modified by the use of a different host system, a point of extreme importance in areas where collaboration between two laboratories is essential.

III. Dissociation of the cold adapted property.

From the values shown in Table 1, it is apparent that the cold recombinant AA-CR13 grew equally well at 39° and 25°C. The line appears to be extremely leaky and this observation seems to contradict earlier data which stipulated that the cold adaptation and temperature sensitivity properties of the cold mutants cannot be dissociated. Hence, studies were initiated with this line to ascertain its characteristics *in vitro* and *in vivo*. A pool of AA-CR13 lines was made in embryonated eggs at 33°C for determination of its growth pattern at different temperatures and its reactogenicity and immunogenicity in ferrets. The

data obtained are summarized in Table II. It is quite evident that the leaky line of virus AA-CR13 grew equally well at both 25° and 39°C when compared with its parents. The cold recombinant in addition did infect ferrets with no observed clinical signs and the line was immunogenic. Further studies to repeat and to evaluate the significance of this finding using different lines of virus is planned for the next contract.

IV. Transfer of cold genes by recombination at 33°C.

The study was undertaken to demonstrate that the acquisition of the "cold" genes in influenza virus is a stable genetic property which can be transferred by recombination at the permissive temperature of 33°C. The data presented in Table III show clearly that transfer of cold genes did take place at 33°C, since 4 clones (Clones No. 2,4,7 and 10) had the antigenic subtype of the wild parent grew equally well at 25°C and 33°C and they were temperature-sensitive. Three clones were revertants (clones 3,6 and 9) while the last three clones were hybrid lines with $N2_{60}$ neuraminidase, and with the acquisition of cold adaptation and temperature-sensitivity. The findings here reinforce our hypothesis that cold mutants can be derived by recombination at the permissive temperature of 33°C by the transfer of the cold genes to the wild parent which is then designated as cold recombinant.

After numerous tries, we were unable to accomplish a recombinational rescue of new surface antigens at 25°C where the cross was between the wild parent rendered inactive by UV-irradiation and the live cold mutant. Similar findings were reported by Spring, et al. in 1975 using ts mutants (5) for rescue of ts genes.

V. Collaborative studies with Drs. Spring, Murphy and Chanock at the National Institute of Allergy and Infectious Diseases.

A. Genetic characterization of the cold variants and ts mutants.

The genetic analysis of 9 ts mutants described at NIH has been previously assigned to 7 complementations groups (6). These nine ts mutants served as prototype strains for the analysis of 5 cold adapted variants and the 12 cold recombinants developed and characterized in this laboratory and made available to the group at NIAID. These cold adapted variants were analyzed by the plate complementation-recombination technique to determine to which complementation group(s) they belong. The results summarized in Table 4 show that all clones of cold adapted and cold recombinant viruses share the lesion represented by complementation group 1. In addition, the cold variants derived from the 1967, 1968 and 1970 strains appear to possess additional lesions. The mechanism of derivation of cold mutants either stepwise, by plaque selection or by recombination at 25°C was subject to some question as it could be argued that the temperature stress selected cold adapted variants from the wild type. This type of selection, however, seems unlikely since simultaneous manipulation and passage of wild type parents alone did not yield cold adapted variants (7). Moreover, when the 10 clones of CR18 are examined it can be seen that six of the recombinants derived their neuraminidase from the cold parent (A/AA/6/60). In addition, the presence of the group 1 lesion in the 4 independently derived cold recombinants of the A/AA/6/60 cold parent would be unlikely on a random basis. It was also demonstrated that cold adaptation induce lesions in other portions of the genome (4).

B. Genetic stablility of a cold recombinant in vivo.

Previous studies of ts vaccine strains in man and hamsters had suggested that appropriate vaccine strains should replicate well in the nasal turbinates of hamster while not undergoing reversion in the lungs of these animals (8). Comparison in the hamsters of cold adapted parent and its recombinants with the 5-FU ts mutants under same condition, has shown that the cold mutants appear to be attenuated. Their genetic stability was correlated with the level of replication in the hamster lung, i.e., viruses with high level of replication showed a tendency to revert to the ts+ phenotype. Two viruses, PI-7 (the parental cold variant) and the CR5 recombinant (A/Queensland/6/72) did not revert in either the lungs or nasal turbinates of hamsters (4). Further comparison of the replication in hamsters of a different cold recombinant CR12 with ts mutants ts-1E and ts-[Clone 13] was undertaken. The CR12 strain was chosen to represent the cold adapted variants since of the 3 cold adapted viruses it grew to the highest titer in the hamster respiratory tract. The data summarized in Table 5 suggests that the CR12 variant is more stable genetically than either the ts-1[E] or ts-[Clone 13] viruses of the NIH group. The low level of reversion observed when the parental cold variant (PI-7 A/AA/6/60) CR6 and CR12 viruses were used to infect hamsters suggested that these viruses might be more gentically stable than ts viruses derived by 5-FU mutagenesis.

Similar results were obtained by Dr. Davenport and his associates using the ferret model to compare the level of replication in lungs and turbinates, and the pathological changes in these tissues as a result of infection with cold recombinant its parents and 2 ts

mutants (see Dr. A.V. Hennessy's progress report, August 1976, Contract No. DADA 17-70-C-0050).

Further investigation is necessary to determine the relative roles of the ts and cold adaptation properties in the attenuation and genetic stability of cold variants and cold recombinants.

C. Characterization of recombinant clones at 33°C.

Studies similar to the one presented in section IV of this report were performed at NIH using a different set of parent strains for recombination at 33°C.

The usual procedure for the transfer of the cold adaptation property is to prepare recombinants at 25°C. It has been suggested that this procedure in itself might select for cold adaptation. Although no evidence has been found for this to date we decided to prepare recombinants of CR12 and A/AA/Marton/43 at 33°C. In addition, the findings that the ts property and cold adapted property appeared to be dissociated in the ts+ revertants isolated from hamsters further prompted these studies (4). The properties of 5 recombinants viruses are presented in Table 6. It is apparent that cold adaptation was transferred at 33°C using the same criteria of plaque morphology and time of appearance of plaques to determine if the recombinant clones were cold adapted. Thus, the data from 2 separate laboratories suggest that temperature sensitivity and cold adaptation can be dissociated during recombination and that neither of these properties is associated with the 2 surface glycoproteins. Further studies along these lines are being undertaken <u>in vitro</u> and <u>in vivo</u>, for possible correlation of these dissociated properties to attenuation.

VI. Testing in man of Clone 7 of the cold recombinant CR18 (A/Scotland/840/74 X A/AA/6/60).

The cold recombinant Clone 7, derived in this laboratory, was characterized biologically in this laboratory and at NIH (see Table III and IV). A vaccine lot was produced and safety tested for use in man by Flow Laboratories. This vaccine lot will be tested by Dr. Brian R. Murphy of NIAID in their Maryland facilities for assessment of its reactogenicity and immunogenicity. Both laboratories will be cooperating in determining virus shedding and the properties of the virus isolated from volunteers in terms of ts and cold adapted characteristics.

VII. Collaborative studies with Dr. A.P. Kendal of the CDC in Atlanta - Biochemical characterization of the cold recombinants.

A high resolution polyacrylamide gel electrophoresis (9) was used to analyze the RNA pattern of the cold recombinant CR6 (A/Queensland/6/72 X A/AA/6/60). The results are shown on the Graph I and II. It is apparent that the CR6 recombinant derived its two smallest genes from the A/AA/6/60 parent. Results of about five experiments are consistent.

Viruses A/AA/6/60 and A/Queensland/6/72 were grown in chick kidney cells in the presence of ^{3}H-uridine. Recombinant CR6 was grown in the presence of ^{32}P. After purification, half of the ^{32}P-labeled CR6 was mixed with each of the parent viruses, for co-electrophoresis on acrylamide gels. The P^{32} and ^{3}H isotope patterns are separately drawn for clarity. Gel 1 shows the difference in sizes between the peaks 7 and 8 of CR6 compared to A/Queensland; whereas gel 2 shows the identity of peaks 7 and 8 in CR6 and A/AA/6/60. Peaks 1, 2, 3, 5 and 6 of these viruses are sufficiently similar in electrophoretic pattern that the derivation of these RNA genes in CR6 cannot be determined from this an-

alysis. However, peak 4 of CR6 is clearly derived from A/Queensland. This concurs with the feeling that peak 4 represents the HA gene (9).

Similar studies are contemplated for other recombinants with differing biological properties. If verified area(s) of the segmented genome of influenza virus responsbile for attenuation can be identified.

FLOW DIAGRAM

DERIVATION OF THE RECOMBINANT AA-CR19-H3N2

(A/VICTORIA/3/75 X A/ANN ARBOR/6/60)

TREATMENT OF "WILD PARENT"	PASSAGE OF THE RECOMBINANT	TREATMENT OF "COLD VARIANT"
A/Victoria/3/75-H3N2	A/Victoria/3/75-H_3N_2 + A/AA/6/60-H_2N_2	A/AA/6/60- H2N2

Passage 1 at 25°C in primary chick kidney tissue culture (PCKTC)
(48-72 hrs.)

Passage 2 and 3 at 25°C in PCKTC with anti A/AA/6/60 immune serum
(5 days each)

Passage 4 in embryonate eggs at 25°C without antiserum
(72 hrs.)

Passage 5-plaque titration at 25°C in PCKTC
(5 days)

No yield	3×10^{-3} PFU/ml.	No yield

Passage 6-plaques (20) picked for separate passage in embryonate eggs at 33°C
(72 hrs.)

Selection of vaccine candidate after biologic and immunologic characterization of yields. These studies are now underway.

TABLE 1

GROWTH OF COLD MUTANTS OF INFLUENZA VIRUS AT DIFFERENT TEMPERATURES

Virus	Method of Selection	Log_{10} reduction* of virus at indicated temperatures from titer observed at 25° C				Cut-off temperature +
		33°	37°	38°	39°	
PI-A/AA/6/60-H2N2	Stepwise	0.4	6.5	7.0	No plaques	37°
A/Aichi/2/68-H3N2	Plaque selection	0.0	2.0	7.5	"	38°
AA-CR6-H3N2 (1972)	Recombination	0.2	4.0	8.0	"	37°
AA-CR12-H3N2 (1973)	"	0.0	1.0	6.0	"	38°
AA-CR13-H3N2 (1973)	"	0.2	0.0	1.5	2.0	39 or
AA-CR18-H3N2 (1974)	"	0.0	1.0	6.0	No plaques	38°

* Titration using the plaque method in primary chick kidney (PCKC)

+ Defined as a 100-fold or greater reduction in plaquing efficiency on PCKC

TABLE II

Properties of the Cold Recombinant AA-CR13-H3N2 In Vitro and In Vivo

Virus	Infectivity titer in PFU/ml		Clinical Signs*	Antibody Response+
	25°	39°		
Wild parent - A/Dun/4/73	0	$2x10^7$	High Fever-Coryza	1024
Attenuated Parent-A/AA/6/60	$8x10^7$	0	None	512
Recombinant-AA-CR13	$5x10^6$	10^6	None	512

* Comparable dose for each of the viruses listed (10^6 PFU/ml.) were given intranasally to each of the 2 ferrets.

+ Antibody titer by HI 21 days after infection. Ferrets were challenged 21 days after infection with 1000 tissue culture dose of the homologous "wild parent". Clinical manifestations were absent and complete protection was evident.

TABLE III

Properties of Recombinant Clones of Influenza Virus Derived at 33°C

Virus	Antigenic Subtype	Infectivity titer PFU*/ml at		
		25°	33°	39°
Wild Parent A/Scot/840/74	$H3_{74}N2_{74}$	No plaques	$3x10^7$	$1x10^7$
Attenuated Parent A/AA/6/60	$H2_{60}N2_{60}$	10^8	$8x10^7$	No plaques
Recombinant-CR18-Clone 1	$H3_{74}N2_{60}$	$8x10^7$	$5x10^7$	No plaques
Clone 2	$H3_{74}N2_{74}$	$5x10^7$	$4x10^7$	No plaques
Clone 3	$H3_{74}N2_{74}$	No plaques	$5x10^7$	$2x10^7$
Clone 4	$H3_{74}N2_{74}$	$2x10^6$	$4x10^6$	No plaques
Clone 5	$H3_{74}N2_{60}$	$3x10^7$	$4x10^7$	No plaques
Clone 6	$H3_{74}N2_{74}$	No plaques	$5x10^7$	$3x10^7$
Clone 7	$H3_{74}N2_{74}$	$8x10^6$	10^7	No plaques
Clone 8	$H3_{74}N2_{74}$	10^7	$3x10^7$	No plaques
Clone 9	$H3_{74}N2_{74}$	No plaques	$5x10^6$	$2x10^6$
Clone 10	$H3_{74}N2_{74}$	$2x10^7$	$3x10^7$	No plaques

* PFU-plaque forming units per ml in primary chick kidney cells.

TABLE IV

TS LESIONS IN COLD VARIANTS AND COLD RECOMBINANTS

Virus	Method of Derivation	Shared Lesions (Complementation Group)[1]	Shut-off Temperature[2]	Antigenic Subtype
Cold Variants				
A/AA/Marton/43-H0N1	Stepwise		>39	$H0_{43}N1_{43}$
A/FM/1/47-H1N1	Stepwise		>39	$H1_{47}N1_{47}$
A/AA/2/65-H2N2	Plaque selection	1	37	$H2_{65}N2_{65}$
A/AA/2/67-H2N2	Plaque selection	1,3,5	37	$H2_{67}N2_{67}$
A/Aichi/2/68-H3N2	Plaque selection	1,6	38	$H3_{68}N2_{68}$
A/AA/1/70-H3N2	Plaque selection	1,3,6		$HE_{68}N2_{68}$
P17-A/AA/60/60-H2N2	Stepwise	1	37	$H2_{60}N2_{60}$
Cold Recombinants				
CR6	P17 X A/Queen/6/72	1	37	$H3_{72}N2_{72}$
CR12	P17 X A/AA/9/73	1	38	$H3_{73}N2_{73}$
CR13	P17 X Dunedin/4/73		>39	$H3_{73}N2_{73}$
CR18 - Clone 1	P17 X A/Scot/840/74	1	38	$H3_{74}N2_{74}$
Clone 2		1		$H3_{74}N2_{74}$
Clone 3		1		$H3_{74}N2_{60}$
Clone 4		1		$H3_{74}N2_{60}$
Clone 5		1	38	$H3_{74}N2_{60}$
Clone 6		1	37	$H3_{74}N2_{74}$
Clone 7		1		$H3_{74}N2_{74}$
Clone 8		1		$H3_{74}N2_{60}$
Clone 9		1	37	$H3_{74}N2_{60}$
Clone 10		1	38	$H3_{74}N2_{60}$

[1] Determined by complementation recobmination assay on RMK monolayers using 5 FU-Hong Kong-ts mutants and recombinants (Spring, et al., 1975, 1976).

[2] Taken as temperature at which there is a 100-fold loss in PFU.

TABLE V

Comparison of Genetic Stability in the Hamster Lung of Cold Recombinant CR 12 and ts Mutants, ts-1[E] and ts-[Clone 13]

Mutant	Shut Off Temp	Maximum Yield ($TCID_{50}$) Day]	Total number of isolates from which Revertant Virus Recovered[a]
CR 12	38	2.7 ± 1.1	1/10[b]
ts-[clone 13]	39	5.0 ± 2.2	10/15[c]
ts-1[E]	38	4.0 ± 2.1	9/14[d]

[a] Although 15 hamsters were inoculated with each virus, not all animals were infected; in addition, some isolates did not yield sufficient virus for titration.

[b] The ratio of PFU 39°C/PFU 34°C was 0.001.

[c] The ratio of PFU 39°C/PFU 34°C was from 0.0001 to 0.1.

[d] The ratio of PFU 39°C/PFU 34°C was from 0.001 to 0.01.

TABLE VI

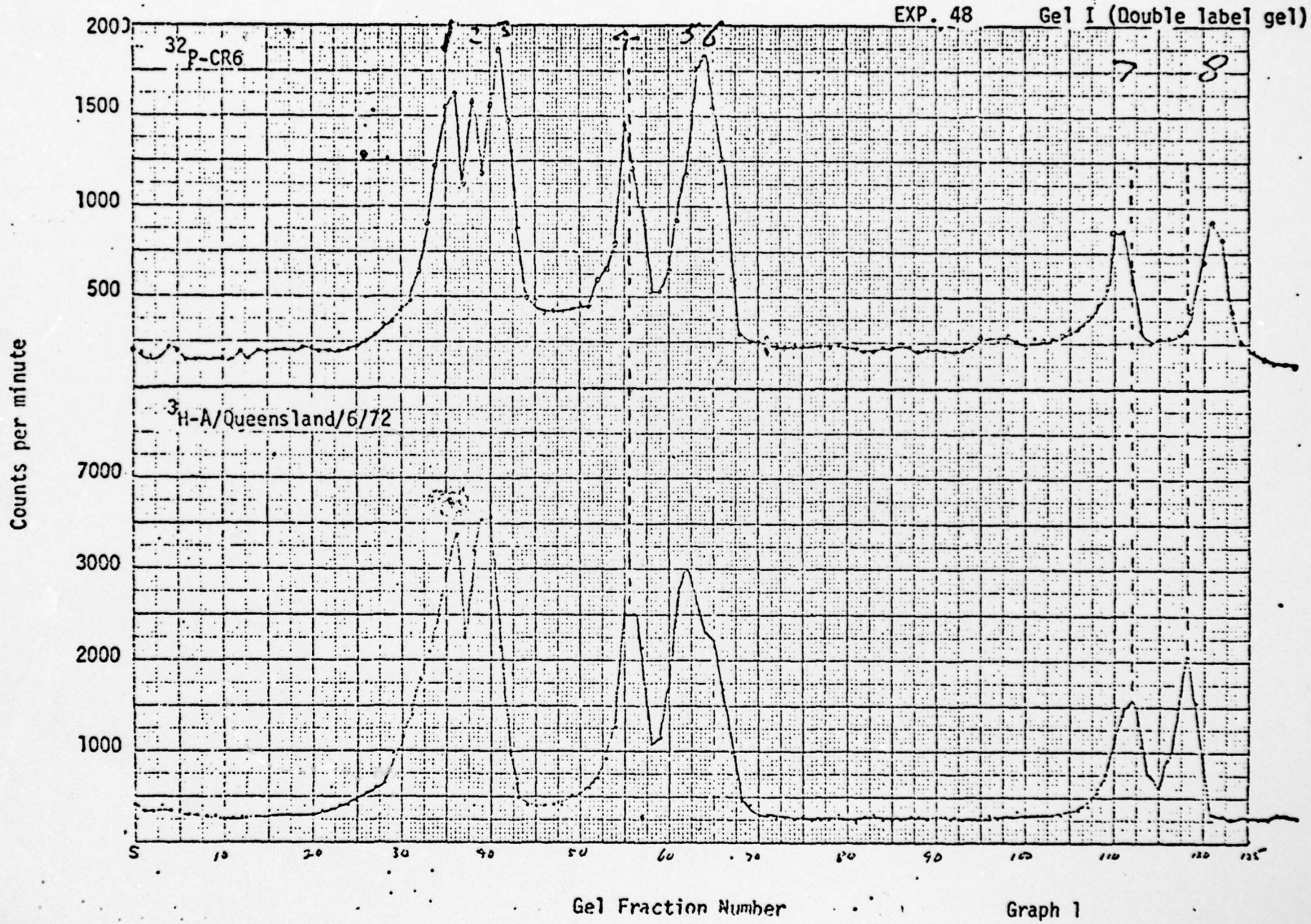
Characterization of Recombinant Clones Derived from a Cross at 33°C of CR-12 and A/AA/Marton/43 Virus

Recombinant Clones	Antigenic Subtype	Titer: PFU/ml				Temperature Sensitivities[b]		Complementation Group if ts
		Chick Kidney		RMK[a]				
		25°C	33°C	33°C	39°C			
804	HON1	5×10^5	1×10^6	5×10^5	$<.00001$	CA	ts	1
18F2	HON1	2×10^6	4×10^6	5×10^6	$<.00001$	CA	ts	1
24A4	HON1	1.1×10^5	2×10^6	1×10^5	2×10^6	CA	ts^+	----
1D2	HON2	1×10^5	6×10^5	1×10^6	3.5×10^5	NCA	ts^+	----
3B3	HON2	1.3×10^6	2×10^6	1×10^6	2×10^5	CA	ts^+	---

[a] RMK = Rhesus monkey kidney.

[b] ts = temperature sensitive; ts^+ phenotypically wild type.

CA = cold adapted; NCA = not cold adapted.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1000
500
7000
3000
2000
1000
1
2
3
4
5
6
7
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Counts per minute
S
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20
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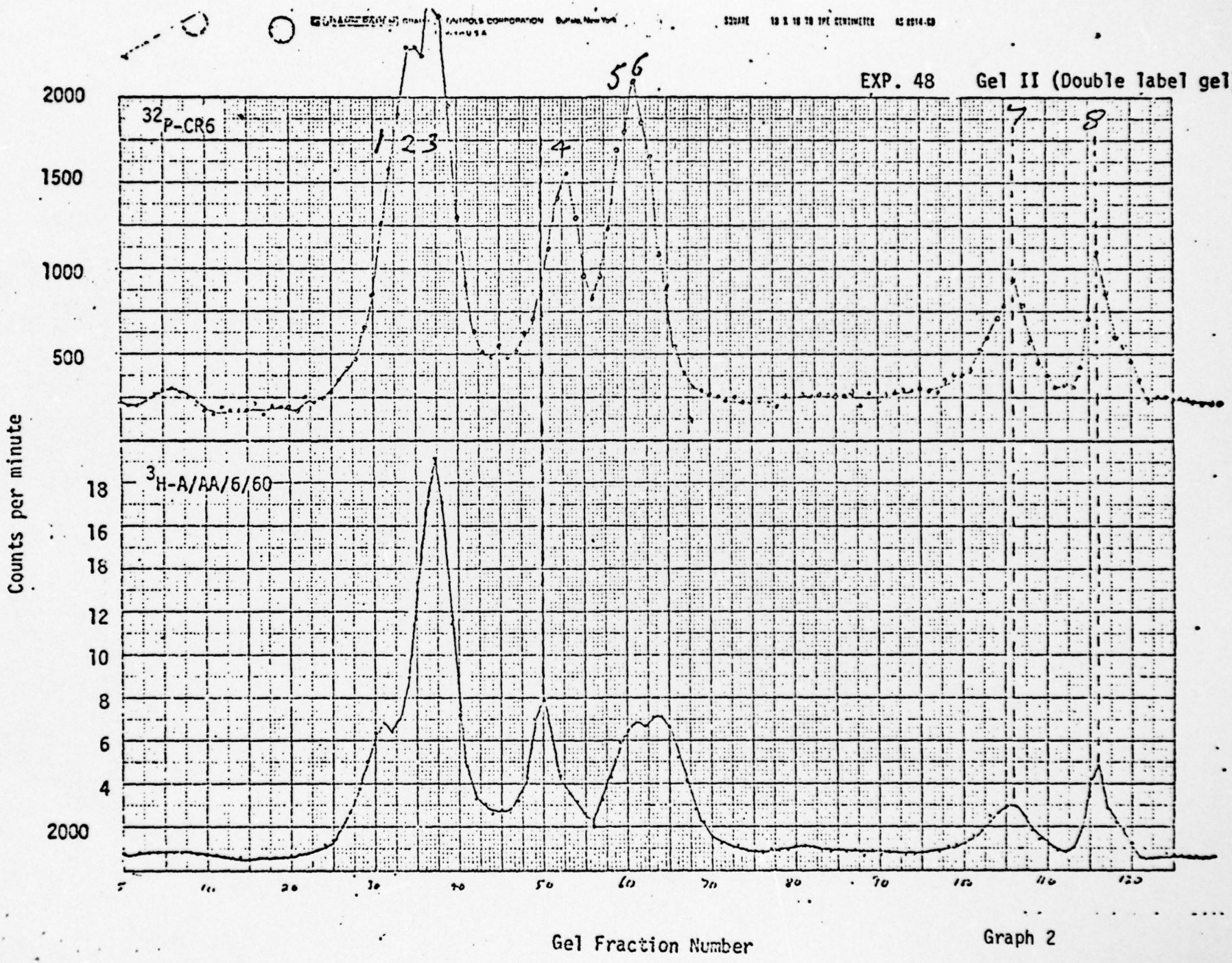
90
100
110
120
125
Gel Fraction Number

Graph 1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^{3}H-A/AA/6/60
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5 6
7
8
2000
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1000
500
18
16
14
12
10
8
6
4
2000
Counts per minute
Gel Fraction Number

Graph 2

REFERENCES

1. Maassab, H.F. (1969). Biologic and immunologic characteristics of cold-adapted influenza virus. J. Immunol. 102:728-752.

2. Maassab, H.F. (1970). Development of variants of influenza virus. In: The Biology of large RNA Viruses (ed. R.D. Barry & B.W.J. Mahy) Acad. Press, p.542.

3. Kitayama, T., Togo, Y., Hornick, R.B. and Friedwald, W.T. (1973). Low-temperature adapted influenza A/AA/6/60 virus vaccines in man. Infec. Immunol. 7:119.

4. Spring, S.B., Maassab, H.F., Kendal, A.P., Murphy, B.R. and Chanock. R.M. (1976). Cold adapted variants of influenza A. I. Comparison of the genetic properties of ts mutants and 5 cold adapted variants of influenza virus A. Virology (submitted for publication).

5. Spring, S.B., Nusinoff, S.R., Mills, J.V., Richman, D.D., Tierney, E.L., Murphy, B.R. and Chanock, R.M. (1975). Temperature-sensitive mutants of influenza virus. VI. Transfer of ts lesions from the Asian subtype of influenza A virus (H2N2) to the Hong Kong, subtype (H3N2). Virology 66:522.

6. Spring, S.B. Nusinoff, S.R., Tierney, E.L., Richman, D.D., Murphy, B.R. and Chanock, R.M. (1975). Temperature-sensitive mutants of influenza virus VIII. Genetic and biological characterization of ts mutants of influenza virus A (H3N2) and their assignment to complementation groups. Virology 66:542.

7. Maassab, H.F. (1975). Properties of influenza virus as cold recombinants. In: Negative strand viruses, edited by B.W.J. Mahy & R.D. Barry. Acad. Press, pp.775-763.

8. Murphy, B.R., Chalhub, E.G., Nusinoff, S.R., Kasel, J. and Chanock, R.M. (1973). Temperature-sensitive mutants of influenza virus. III. Further characterization of the ts-1E influenza A recombinant (H3N2) in man. J. Infect. Dis. 128:479.

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